# Defensin

### Week 15

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

## Summary

**1 Addition of BamHI restriction site downstream to the defensin sequence and amplification of the whole plasmid, using overhang PCR 2**

**2 Addition of BamHI restriction site downstream to the defensin sequence and amplification of the whole plasmid, using overhang PCR - second attempt 4**

**3 Transformation of pSB1C3-T7-Def in Top10 and BL21(DE3) with inoculation in liquid medium 8**

**4 Addition of BamHI restriction site downstream to the defensin sequence and amplification of the whole plasmid, using overhang PCR - third attempt 9**

# 1 Addition of BamHI restriction site downstream to the defensin sequence and amplification of the whole plasmid, using overhang PCR

## Responsible

Oscar He

## Protocols used

PCR Amplification (Phusion)
Gel electrophoresis

## Modifications and comments to protocols

| | |
|---|---|
| PCR Amplification (Phusion) primers: | Def_R (reverse)<br>Suffix_F (forward) |
| PCR Amplification (Phusion) buffer: | GC Buffer |

# Experimental Set Up

Table 1: Concentration of samples used for the ovarhang PCR amplification.

| Sample | Concentration [ng/$\mu$l] |
|---|---|
| pSB1C3-T7-Def (diluted from 66.7 ng/$\mu$l) | 33.3 |
| pSB1C3-T7-Def | 66.7 |

Table 2: Volumes of components in PCR mix.

| Component | Volume $\mu$l] |
|---|---|
| 10 $\mu$M Suffix_F | 1 |
| 10 $\mu$M Def_R | 1 |
| 5X Phusion GC Buffer | 4 |
| Phusion DNA Polymerase | 0.2 |
| 2 mM dNTP mix | 2 |
| Template DNA | 1 |
| dd$H_2O$ | 10.8 |

Table 3: Thermocycling conditions, with two different annealing temperatures.

| Step | Temperature [°C] | Time [sec] |
|---|---|---|
| Initial Denaturation | 98 | 30 |
| 35 x | 98 | 10 |
| | 65 | 30 |
| | 72 | 60 |
| Final Extension | 72 | 600 |
| Hold | 4 | N/A |

## Results and Conclusions

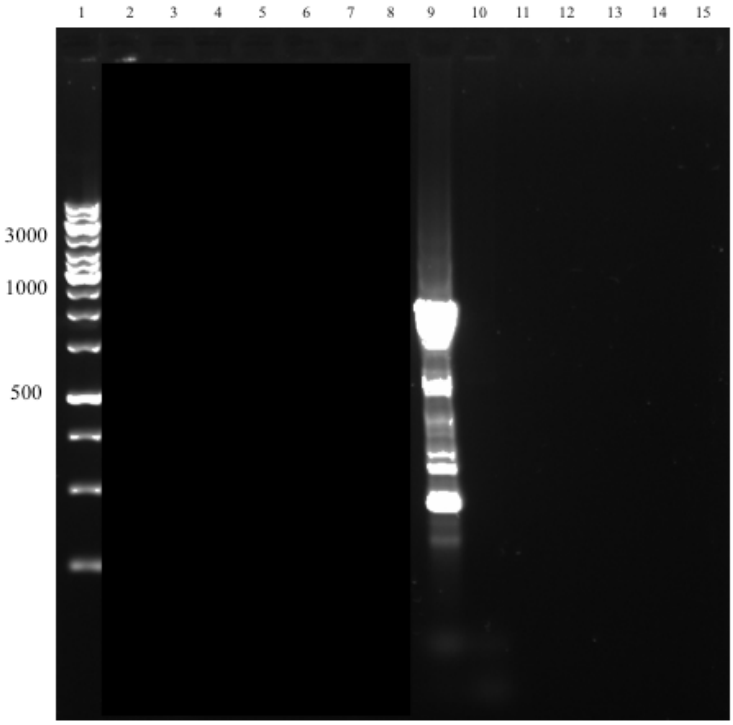


Figure 1: Result of the gel electrophoresis of overhang PCR product, with 1 % agarose at 110 V. (1) DNA Ladder (9) Def 66.7 ng/$\mu$l (10) Def 33.3 ng/$\mu$l.

## Discussion and Troubleshooting

The PCR product contained a lot of contaminations at smaller sizes. Further attempts will be performed at higher annealing temperatures. Also, DMSO will be added as one of two duplicates to eliminate any suspicions regarding difficulties in denaturing the template plasmid.

# 2 Addition of BamHI restriction site downstream to the defensin sequence and amplification of the whole plasmid, using overhang PCR - second attempt

### Responsible

Oscar He

### Protocols used

PCR Amplification (Phusion)
Gel electrophoresis

### Modifications and comments to protocols

| | |
|---|---|
| PCR Amplification (Phusion) primers: | Def_R (reverse)<br>Suffix_F (forward) |
| PCR Amplification (Phusion) buffer: | GC Buffer |

## Experimental Set Up

Table 4: Concentration of samples used for the ovarhang PCR amplification.

| Sample | Concentration [ng/$\mu$l] |
|---|---|
| pSB1C3-T7-Def | 66.7 |

Table 5: Volumes of components in PCR mix (without DMSO).

| Component | Volume $\mu$l] |
|---|---|
| 10 $\mu$M Suffix_F | 1 |
| 10 $\mu$M Def_R | 1 |
| 5X Phusion GC Buffer | 4 |
| Phusion DNA Polymerase | 0.2 |
| 2 mM dNTP mix | 2 |
| Template DNA | 1 |
| dd$H_2O$ | 10.8 |

Table 6: Volumes of components in PCR mix (with DMSO).

| Component | Volume $\mu$l] |
|---|---|
| 10 $\mu$M Suffix_F | 1 |
| 10 $\mu$M Def_R | 1 |
| 5X Phusion GC Buffer | 4 |
| Phusion DNA Polymerase | 0.2 |
| 2 mM dNTP mix | 2 |
| Template DNA | 1 |
| DMSO | 0.6 |
| $ddH_2O$ | 10.2 |

Table 7: Thermocycling conditions, with two different annealing temperatures.

| Step | Temperature [°C] | Time [sec] |
|---|---|---|
| Initial Denaturation | 98 | 30 |
| 35 x | 98 | 10 |
| | 65 | 30 |
| | 72 | 60 |
| Final Extension | 72 | 600 |
| Hold | 4 | N/A |

# Results and Conclusions

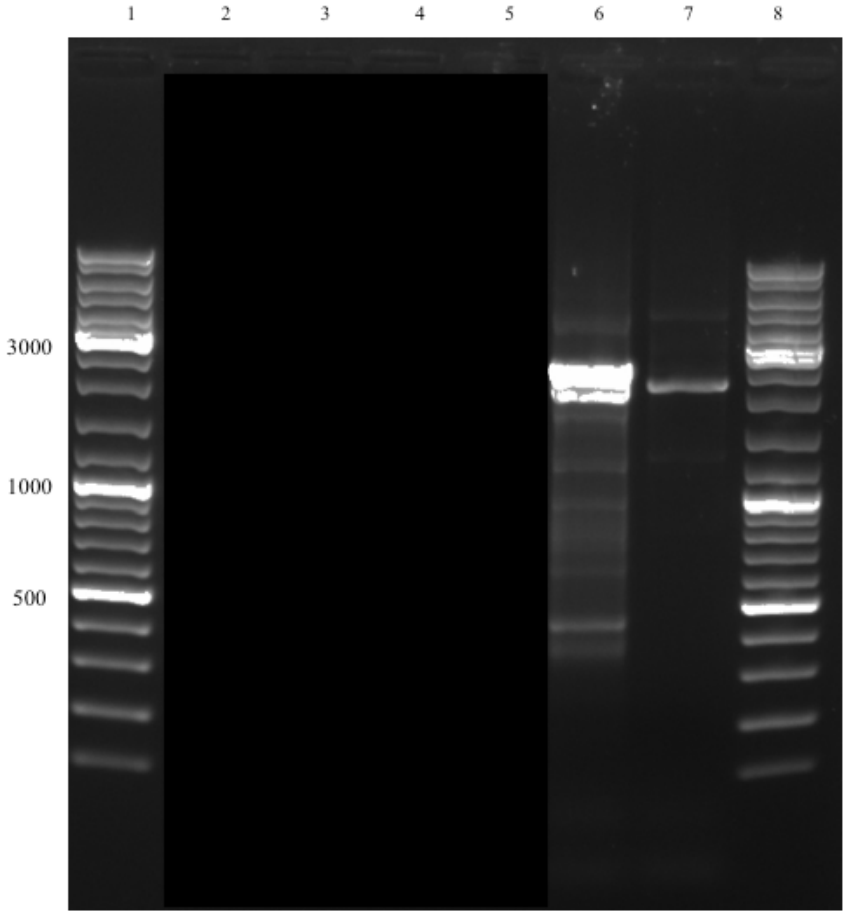


Figure 2: Result of the gel electrophoresis of overhang PCR product, with 1 % agarose at 110 V. (1) DNA Ladder (6) Def (7) Def DMSO (8) DNA Ladder.

## Discussion and Troubleshooting

Since the PCR was successful, the Def product with added DMSO will be used for further experiments. There are tiny contaminations and a third attempt will therefore be performed with a slightly higher annealing temperature just to try to eliminate those contaminations.

# 3 Transformation of pSB1C3-T7-Def in Top10 and BL21(DE3) with inoculation in liquid medium

### Responsible

Ellinor Lindholm

### Protocols used

Transformation
Colony picking

## Experimental Set Up

Two cells from each plate were picked and inoculated in liquid media.

Table 8: Volumes of liquid culture for each picked colony.

| Sample | Volume [$\mu$l] |
|---|---|
| Def Top10 1 | 5 |
| Def Top10 2 | 5 |
| Def BL21(DE3) 1 | 5 |
| Def BL21(DE3) 2 | 5 |

## Results and Conclusions

Successful, a lot of cells for both Top10 and BL21(DE3).

# 4 Addition of BamHI restriction site downstream to the defensin sequence and amplification of the whole plasmid, using overhang PCR - third attempt

## Responsible

Oscar He

## Protocols used

PCR Amplification (Phusion)
Gel electrophoresis

## Modifications and comments to protocols

| | |
|---|---|
| PCR Amplification (Phusion) primers: | Def_R (reverse)<br>Suffix_F (forward) |
| PCR Amplification (Phusion) buffer: | GC Buffer |
| PCR Amplification (Phusion) annealing temperature: | 66 °C |

# Experimental Set Up

Table 9: Concentration of samples used for the ovarhang PCR amplification.

| Sample | Concentration [ng/$\mu$l] |
|---|---|
| pSB1C3-T7-Def | 66.7 |

Table 10: Volumes of components in PCR mix (with DMSO).

| Component | Volume $\mu$l] |
|---|---|
| 10 $\mu$M Suffix_F | 1 |
| 10 $\mu$M Def_R | 1 |
| 5X Phusion GC Buffer | 4 |
| Phusion DNA Polymerase | 0.2 |
| 2 mM dNTP mix | 2 |
| Template DNA | 1 |
| DMSO | 0.6 |
| dd$H_2O$ | 10.2 |

Table 11: Thermocycling conditions, with two different annealing temperatures.

| Step | Temperature [°C] | Time [sec] |
|---|---|---|
| Initial Denaturation | 98 | 30 |
| 35 x | 98 | 10 |
| | 66 | 30 |
| | 72 | 60 |
| Final Extension | 72 | 600 |
| Hold | 4 | N/A |

## Results and Conclusions

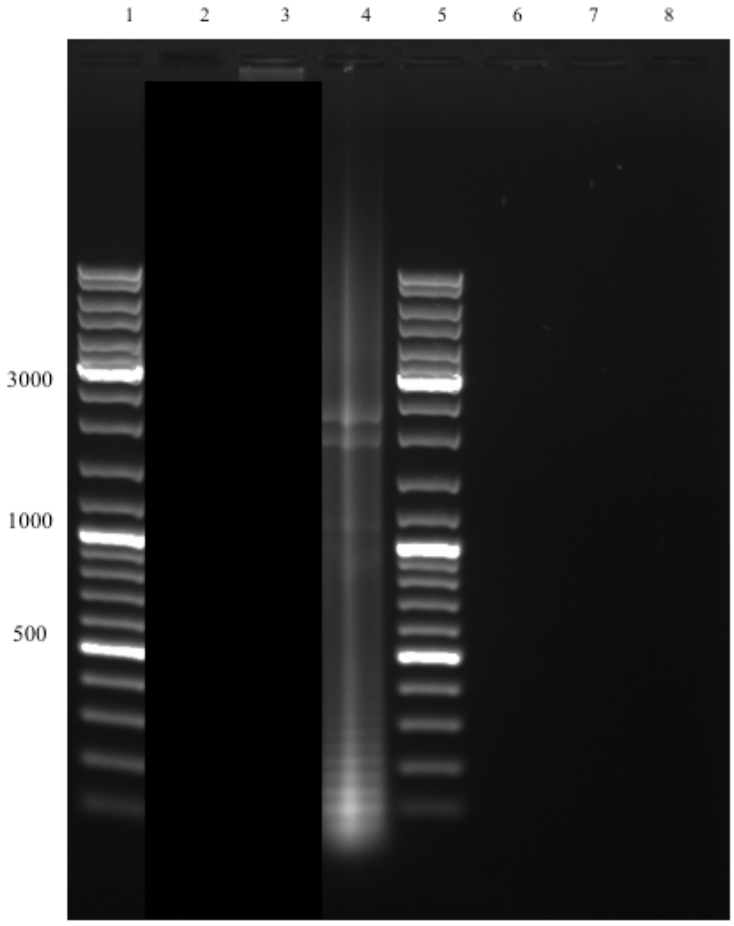


Figure 3: Result of the gel electrophoresis of overhang PCR product, with 1 % agarose at 110 V. (1) DNA Ladder (4) Def (5) DNA Ladder.

## Discussion and Troubleshooting

The result from the gel were hard difficult to interpret. Due to the limited time of the project and the upcoming wiki freeze, no further attempts of refining the PCR protocol will be made. The PCR protocol used in the second attempt will be the one used for amplifying product for further experiments.